Branched cells in the prothallium of Onoclea sensibilis L.

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(WITH PLATES 22 AND 23)

The fern gametophyte offers an interesting field for experimental culture work, not only in regard to the reproductive organs as various writers have shown, but in the germination of the spore and the development of the prothallium. Material is readily procured and the growing conditions may be easily controlled. The average fern spore will germinate on water or moist soil and the prothallium lives about three or four months or until a sporophyte has been established. Many have been known to live much longer.

The normal development of the gametophyte of different ferns has been described by various writers. In a recent research on the prothallium of *Camptosorus rhizophyllus* (L.) Link, Pickett (1) found that the prothallia showed a wide variation in size and form. The apical group is usually unsymmetrically placed and the typical wedge shaped apical cell is rarely seen. Old prothallia were found with several marginal growing regions and proliferations capable of independent growth were produced. In some very interesting experiments with this fern Pickett (2) had already shown that the drought resisting character of these prothallia was an efficient factor in the establishing of this fern to its habitat.

The dioecious prothallia of *Onoclea Struthiopteris* (L.) Hoffm. have been the subject of a physiological problem by Miss Wuist, (3) who has obtained monoecious forms in this fern by growing prothallia in culture solutions. Monoecious prothallia were also observed in soil cultures. Miss Wuist mentions the appearance of irregular and filamentous forms, some exceedingly long, in these cultures. Mottier (4) working with the same fern finds that about 12 per cent. of the prothallia are monoecious under optimum culture conditions. Branched filamentous prothallia have been described by Miss Pace (5) in fern gametophytes. Many examples

of the retention of the filamentous form of gametophyte due to feeble light and poor nutritive conditions and even of a reversion to the formation of cell threads, after an expanded prothallium had been established when nutritive conditions became unfavorable are given in Goebel's Organography (**6**, pp. 202–205).

Some very irregular filamentous prothallia, evidently the result of feeble light and poor nutritive conditions, were found by the writer in an old culture of *Onoclea sensibilis* L. A rather unusual character of these prothallia was the tendency of certain cells to branch. It was thought that a description of them might be of interest to others investigating fern prothallia.

Spores of *Onoclea sensibilis* were sown upon sterile distilled water for class use. Through oversight, the covered glass dish containing the culture was left undisturbed in somewhat dim light for approximately six weeks. When it was then observed the culture appeared green and healthy. Upon examining the culture, part of the prothallia were floating on the surface of the water and some smaller clumps were entirely submerged near the bottom of the dish. Slides were prepared from both sources. The prothallia taken from the surface presented the typical form with a well-defined apical region as shown in FIG. 1. Some of them consisted of thirty to fifty cells and bore a few antheridia.

The submerged prothallia were found to differ considerably from the normal gametophyte. In these prothallia the cells were greatly elongated and contained few chloroplasts scattered throughout. It was of interest to note that many cells had produced one or more outgrowths without cutting off the usual cross wall. FIG. 2 shows a five-celled prothallium, the last two cells of which are unusually long. The enlarged end of the apical cell is turned, indicating an abrupt change in the direction of growth. In FIG. 3 a small cell arises from the elongated apical cell. This cell is so turned that it is growing in the opposite direction from the main apical cell. This cells resembles a rhizoid except for its size and the presence of chlorophyll. In FIG. 4, the end of the filament forms an acute angle with the main cell-thread. This is due to a change in the direction of growth of the apical cell. A very small outgrowth is cut off laterally from the apical cell. The abrupt turning of the branches is not caused by contact with

the wall of the glass dish as none of the submerged prothallia examined were observed near the side of the dish. Fig. 5 seems a more normal prothallium having average sized cells. The end of the filament is turned so that there is a possibility of two growing regions here.

An extreme case of the tendency to form branched cells is seen in FIG. 6. Two cells of this filament have well defined protuberances, one of which is considerably elongated. The tip of the apical cell is also somewhat curved. A two-armed prothallium is seen in FIG. 7, due to the branching of one cell. This is an unusually long filamentous prothallium. The shorter branch consists of two cells. FIGS. 8 and 9 show great variety in the apical cell, each having a number of lobes. This suggests considerable diversity in the subsequent growth of the prothallium. In each case the apical cell is curved with two lobes at the extreme tip. FIG. 10 shows a young stage of a two-armed prothallium. In FIG. 11, three branches are found with their origin in one cell. Two of these branches are limited by cross walls. The increased activity of the cells where one or more branches are produced may be explained by considering that the active cell was once the apical cell and in a normal prothallium would remain the center of growth.

In sowing fern spores it is not unusual to find sporangia or a fragment of leaf in the culture. It was thought that an attached sporangium or bit of leaf caused the sinking of some of the spores, thus changing their environment by completely surrounding the developing prothallia with water. The dish was covered with a loosely fitting lid so that prothallia growing on the surface of the water would receive a sufficient supply of oxygen. The amount of oxygen would necessarily be limited in the prothallia growing below the surface of the water. It is suggestive that the oxygen requirement may have been a factor in producing these irregular gametophytes.

Besides the filament of elongated cells characteristic of impoverished prothallia, the accompanying drawings show that these prothallia differ from the normal gametophyte in one or more of the following characteristics:

1. A change in the direction of growth of the filament may occur by the apical cell forming an acute angle with the main cell thread (FIG. 4).

2. The production of an irregularly lobed apical cell (FIGS. 2, 8, 9).

3. An individual cell in the filament may develop an outgrowth or branch without forming a cross wall at the base (FIGS. 6, 7, 10).

4. A branched filamentous prothallium may have the proliferations originating in one cell (FIGS. 7, 10, 11).

5. An increase in the number of growing regions. (FIGS. 3–11).

An exact knowledge of the optimum physiological conditions under which fern prothallia will grow and the changes that occur when this condition is modified would be of interest since the fern gametophyte has shown itself to possess great potentiality.

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Explanation of plates 22 and 23

All the figures were drawn with the aid of a camera lucida.

FIG. 1. Normal prothallium from the surface of culture showing typical form with a well-defined apical region.

FIG. 2. A five-celled prothallium with an unusually long apical cell, swollen at the end, which is turned, indicating an abrupt change in the direction of growth.

FIG. 3. The end of the filament forms an acute angle with the main cell thread.

FIG. 4. A small cell cut off from the apical cell is growing in the opposite direction.

FIG. 5. A normal gametophyte with the possibility of two growing points.

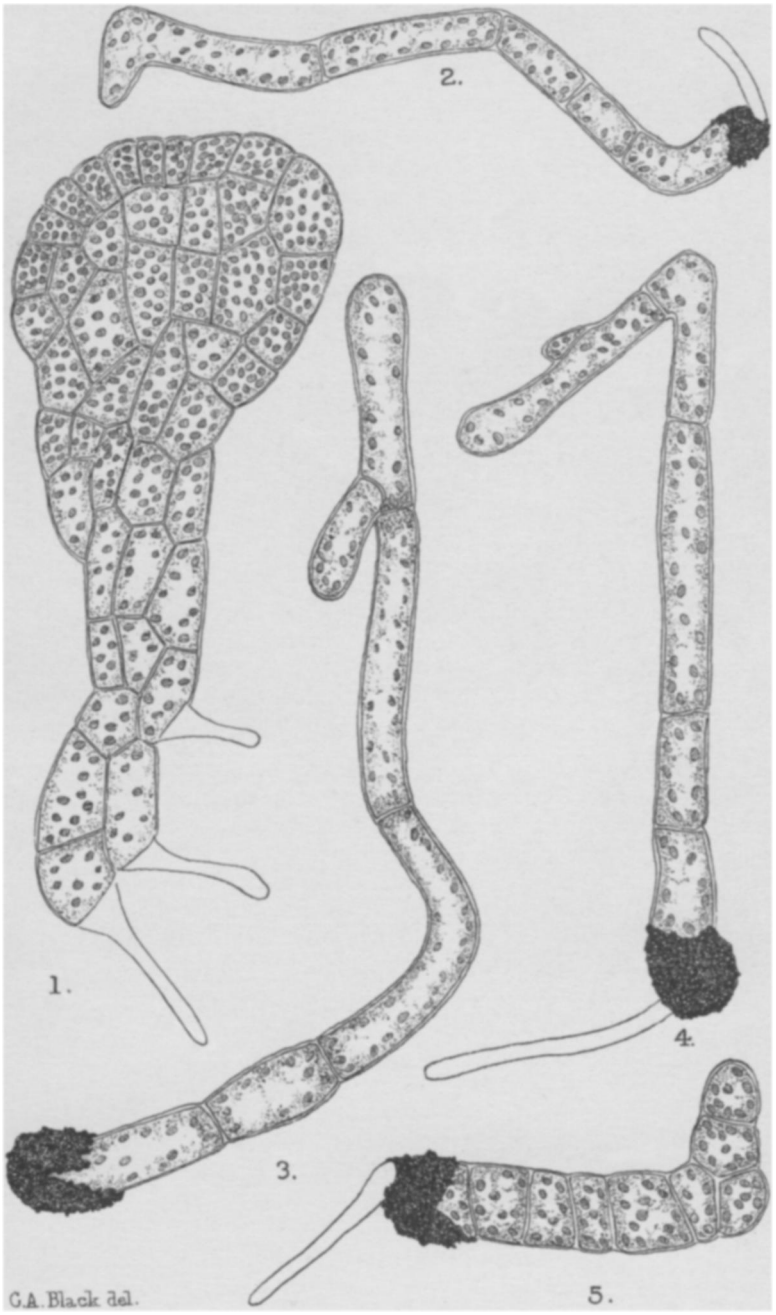
FIG. 6. An extreme case of branched cells; two cells have well-defined protuberances.

FIG. 7. Two filaments originating in a two-armed cell.

FIGS. 8, 9. Irregularly lobed apical cells.

FIG. 10. A two-armed prothallium due to the branching of one cell.

FIG. 11. Three branches originating in one cell.



BLACK: PROTHALLIUM OF ONOCLEA SENSIBILIS L.

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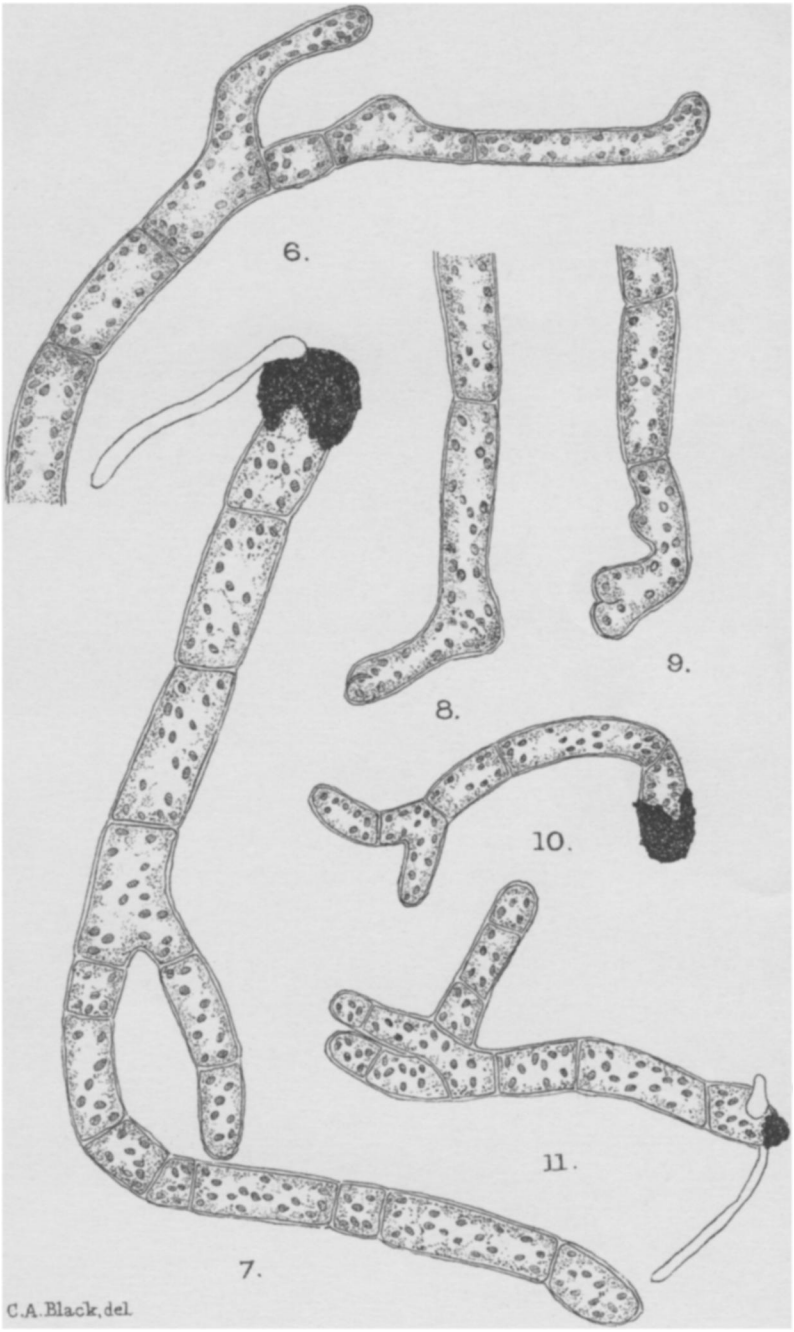

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